(Chel)Nle<u>DHF$_d$ RWK</u>-NH_2, (SEQ ID NO:1)

Ac-HSDAVFTENYTKLRK(Chel)QNleAAK<u>KYLND</u>LKKGGT-NH_2, (SEQ ID NO:10)

(Chel)γAbuHSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2, (SEQ ID NO:2)

(Chel)γAbuVFTDNYTRLRKQMAVKKYLNSILN-NH_2, (SEQ ID NO:4)

(SEQ ID NO:1) (Chel)γAbuNle<u>DHF$_d$ RWK</u>-NH_2[c], <GHWSYK(Chel)LRPG-NH_2, (SEQ ID NO:6)

(SEQ ID NO:7) <GHYSLK(Chel)WKPG-NH_2, Ac<u>Nal$_d$ CPa$_d$ W$_d$ SRK$_d$</u> (Chel)LRPA$_d$ –NH_2, (SEQ ID NO:8)

(SEQ ID NO:11) <GHYSYLK(Chel)WKPG-NH_2, <GHYSLK(Chel)WKPG-NH_2, (SEQ ID NO:9)

(SEQ ID NO:12) <u>Nal$_d$ Cpa$_d$ W$_d$ SRK$_d$</u> (Chel)WKPG-NH_2, <GHWSYK$_d$ (Chel)LRPG-NH_2, (SEQ ID NO:13)

Ac<u>Nal$_d$ Cpa$_d$ W$_d$ SRK$_d$</u> (Chel)LRPA$_d$ –NH_2, (SEQ ID NO:8) Ac<u>Nal$_d$ Cpa$_d$ W$_d$ SRK$_d$</u> (Chel)LRPA$_d$ –NH_2, (SEQ ID NO:8)

(SEQ ID NO:8) Ac<u>Nal$_d$ Cpa$_d$ W$_d$ SRK$_d$</u> (Chel)LRPA$_d$–NH_2, <GHWSYK(Chel)LRPG-NH_2, (SEQ ID NO:6)

(SEQ ID NO:14) AcK(Chel)<u>F$_d$ CFW$_d$ KTCT</u>-OH, AcK(Chel)<u>DF$_d$ CFW$_d$ KTCT</u>-OH, (SEQ ID NO:15)

(SEQ ID NO:14) AcK(Chel)<u>F$_d$ CFW$_d$ KTCT</u>-ol, AcK(Chel)<u>DF$_d$ CFW$_d$ KTCT</u>-ol, (SEQ ID NO:15)

(SEQ ID NO:16) (Chel)<u>DF$_d$ CFW$_d$ KTCT</u>-OH, K(Chel)<u>DF$_d$ CFW$_d$ KTCT</u>-ol, (SEQ ID NO:15)

(SEQ ID NO:17) K(Chel)KK<u>F$_d$ CFW$_d$ KTCT</u>-ol, K(Chel)K<u>DF$_d$ CFW$_d$ KTCT</u>-OH, (SEQ ID NO:18)

(SEQ ID NO:19) K(Chel)<u>DSF$_d$ CFW$_d$ KTCT</u>-OH, K(Chel)<u>DF$_d$ CFW$_d$ KTCT</u>-OH, (SEQ ID NO:15)

(SEQ ID NO:20) K(Chel)<u>DF$_d$ CFW$_d$ KTCD</u>-NH_2, K(Chel)<u>DF$_d$ CFW$_d$ KTCT</u>-NH_2, (SEQ ID NO:15)

(SEQ ID NO:18) K(Chel)K<u>DF$_d$ CFW$_d$ KTCT</u>-$NHNH_2$, AcK(Chel)<u>F$_d$ CFW$_d$ KTCT</u>-$NHNH_2$, (SEQ ID NO:14)

(SEQ ID NO:14) K(Chel)<u>F$_d$ CFW$_d$ KTCT</u>-ol, and <u>F$_d$ CFW$_d$ KTCT</u>K(Chel)-NH_2, (SEQ ID NO:21)

wherein (Chel) is a radiometal-binding moiety having the structure set forth above.

Page 24, lines 2 - 3

Naturally occurring VIP has the sequence:

HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO:2)

Page 24, lines 10-34

Chelating derivatives based on attachment of the metal binding ligand at these positions include, but are not limited to, those with a metal binding moiety attached, either directly or via a spacer group, to the pharmacophore via the side chain amine of a lysine or other bis-amino acid residue. Specific chelating derivatives of this general structure include, but are not limited to:

MaGCγAbuHSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO:2)

AcCGCHSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO:22)

KPRRPYTDNYTRLRK(PtscGC)QMAVKKYLNSILN-NH_2 (SEQ ID NO:3)

B3 MaGCγAbuVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO:4)

AcCGCVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO:23)

MaGCγAbuYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO:5)

HSDAVFTDNYTRLRK(PtscGC)QMAVKKYLNSILN-NH_2 (SEQ ID NO:2)

HSDAVFTDNYTRLRK(Dtpa)QMAVKKYLNSILN-NH_2 (SEQ ID NO:2)

HSDAVFTDNYTRLRK(AGC)QMAVKKYLNSILN-NH_2 (SEQ ID NO:2)

where Ma is mercaptoacetic acid,

PtscG is 2-(4-phenyl-3-thiosemicarbazidyl)acetic acid or PhNHCSNHNHCH$_2$ CO_2 H,

γAbu is γ-aminobutyric acid, and

in K(PtscGC), the parentheses denote that enclosed amino acids are attached to the .epsilon. amine of lysine and the first amino acid attached is C followed by PtscG.

Page 25, lines 2-14:

Naturally occurring LHRH has the sequence:

<GHWSYGLRPG-NH_2 (SEQ ID NO:24)

where <G is pyroglutamic acid. It is further known that the bicyclic peptide

AcNal_d Cpa_d W_d (cyclo4-10)D(cyclo5-8)ER_d LKPDap-NH_2 (SEQ ID NO:25)

B4 (where W_d indicates that the D isomer of the amino acid was used, Nal is 2-naphthylalanine, Cpa is 4-chlorophenylalanine and Dap is 2, 3-diaminopropionic acid) binds to the LHRH receptor. See Bienstock *et al. J. Med. Chem.* 36:3265 (1993). It is also known that the side chain of position 6 of LHRH is very bulk tolerant. See Barbacci *et al. J. Biol. Chem.* 270:9585 (1995). This location is a possible site for the attachment of a metal binding ligand according to the present invention.

Page 25, line 16 to page 26, line 33:

Linear chelating derivatives based on attachment of the metal binding ligand at this position include, but are not limited to, those with a metal binding moiety attached, either directly or via a spacer group, to the pharmacophore via the side chain amine of a lysine or other bis-amino acid residue. Specific linear chelating derivatives of these general structures include, but are not limited to:

<GHWSYK(MaGC)LRPG-NH_2 (SEQ ID NO:6)

<GHYSLK(MaGC)WKPG-NH_2 (SEQ ID NO:7)

<GHWSYK(Ma-azaGC)LRPG-NH_2 (SEQ ID NO:6)

<GHYSLK(PtscGC)WKPG-NH_2 (SEQ ID NO:7)

<GHYSLK(PtscGDap)WKPG-NH_2 (SEQ ID NO:7)

<GHWSYK$_d$(MaGC)LRPG-NH_2 (SEQ ID NO:13)

<GHYSLK(azaGGC)WKPG-NH_2 (SEQ ID NO:7)

<GHWSYK(iECG)LRPG-NH_2 (SEQ ID NO:6)

<GHWSYK$_d$(MtaGC$_a$)LRPG-NH_2 (SEQ ID NO:13)

<GHYSLK(iECiD)WKPG-NH_2 (SEQ ID NO:7)

<GHYSLK(DiGlyGDap)WKPG-NH_2 (SEQ ID NO:7)

<GHYSLK(iDGDap)WKPG-NH_2 (SEQ ID NO:7)

<GHWSYK(MtaGC)LRPG-NH_2 (SEQ ID NO:6)

<GHWSK(MaGC)W$_d$ LRPG-NH_2 (SEQ ID NO:26)

<GHWSYK$_d$ (MtaGDap)LRPG-NH_2 (SEQ ID NO:13)

<GHWSYK$_d$ (PtscGC)LRPG-NH_2 (SEQ ID NO:13)

<GHWSYK$_d$ (E)LRPG-NH_2 (SEQ ID NO:13)

<GHWSYK$_d$ (MtscGC)LRPG-NH_2 (SEQ ID NO:13)

<GHWSYK$_d$ (Mta(hqss)GDap)LPG-NH_2 (SEQ ID NO:8)

AcNal$_d$ Cpa$_d$ W$_d$ SRK$_d$ (MaGC)LRPA$_d$ –NH_2 (SEQ ID NO:8)

Nal$_d$ Cpa$_d$ W$_d$ SRK$_d$ (PtscGC)LRPA$_d$ –NH_2 (SEQ ID NO:8)

AcNal$_d$ Cpa$_d$ W$_d$ SRK$_d$ (MaFC)LRPA$_d$ – NH_2 (SEQ ID NO:8)

AcNal$_d$ Cpa$_d$ W$_d$ SRK$_d$ (azaGFC)LRPA$_d$ – NH_2 (SEQ ID NO:6)

where:

<G is pyroglutamic acid,

Ma is mercaptoacetic acid

azaG is azaglycine or H_2 NNHCH$_2$ CO_2 H,

PtscG is 2-(4-phenyl-3-thiosemicarbazidyl)acetic acid or PhNHCSNHNHCH$_2$ CO_2 H,

Dap is 2, 3-diaminopropionic acid

iD is an aspartic acid coupled via the side chain carboxyl group,

iE is a glutamic acid coupled via the side chain acid group,

DiGly is HOOCCH$_2$ NHCH$_d$ COO-,

Mta(hqss) is S-(2,5-dihydroxyphenyl-S-methyl) sulfoniumacetyl

C_a is an Acm protected cysteine

Mta is the methylthioether of mercaptoacetic acid,

Nal is 2-naphthylalanine,

Cpa is 4-chlorophenylalanine,

in K_d, the subscript d denotes that the D isomer was used, and

in K(MaGC), the parentheses denote that enclosed amino acids are attached to the ε amine of lysine and the first amino acid attached is C followed by G and ending in Ma.

Page 26, line 34 to page 27, line 2:

Additionally, complexes of these peptides with non-radioactive metals may be prepared. Such complexes include:

B6

<GHWSYK(MaGC)LRPG-NH$_2$ ReO

<GHYSLK(MaGC)WKPG-NH$_2$ ReO (SEQ ID NO:7)

<GHYSLK$_d$ (MaGC)LRPG-NH$_2$ ReO (SEQ ID NO:27)

Page 27, lines 7-8:

B7

Naturally occurring α-MSH has the sequence:

Ac-SYSMEHFRWGKPV-NH$_2$ (SEQ ID NO:28).

Page 27, lines 9-19:

B8

It had previously been shown that the cyclic peptide Nle<u>DHF$_d$ RWK</u>-NH$_2$ (SEQ ID NO:1) (where Nle is norleucine and F_d indicates D-Phe) has a high affinity for the α-MSH receptor and is known to be relatively stable *in-vivo*. See Al-Obeidi *et al. J. Amer. Chem. Soc.* 111:3413 (1989); Haskell-Luevano *et al. J. Med. Chem.* 39:432 (1996). The underlined portion indicates those

residues within the cyclized portion of the peptide, and also the termini of the cyclic structure, i.e. the peptide is cyclized by an amide bond from the side chains of aspartic acid and lysine.

Page 27, lines 20-30:

Linear chelating derivatives based upon the structures of these known α-MSH receptor binding peptides include those with a chelating derivative attached to the N-terminus of the peptide, either directly or via a spacer group, such as γ-amino butyric acid (γ-Abu). Specific linear chelating derivatives with this general structure include, but are not limited to:

B_9

MaGCγAbuSYSNleDHF$_d$ RWK-NH$_2$, (SEQ ID NO:9) and

MaGCγAbuSYSNleDHF$_d$ R$_n$ WK-NH$_2$ (SEQ ID NO:29)

where γ-Abu is γ-aminobutyric acid and R$_n$ is a nitrated arginine residue.

Page 33, lines 2-15:

Naturally occurring α-MSH has the sequence Ac-SYSMEHFRWGKPV-NH$_2$ (SEQ ID NO:28). It had previously been shown that the cyclic peptide Nle<u>DHF$_d$ RWK</u>-NH$_2$ (SEQ ID NO:1) (where Nle is norleucine and F$_d$ indicates D-Phe) has a high affinity for the α-MSH receptor and is known to be relatively stable *in-vivo*. See Al-Obeidi *et al. J. Amer. Chem. Soc.* 111:3413 (1989); Haskell-Luevano *et al. J. Med. Chem.* 39:432 (1996). The underlined portion indicates those residues within the cyclized portion of the peptide, and also the termini of the cyclic structure, i.e. the peptide is cyclized by an amide bond from the side chains of aspartic acid and lysine. This cyclic structure is used as a basis for constructing labeled peptides according to the present invention.

B_{10}

Page 33, lines 16-32:

Cyclic chelating derivatives based upon the structure of the known α-MSH receptor binding ligand include those with a chelating derivative attached to the N-terminus of the peptide, either directly or via a spacer group, such as γ-amino butyric acid (γ-Abu). Specific chelating derivatives of this general structure include, but are not limited to:

B_{11}

MaGCγAbuNle<u>DHF$_d$ RWK</u>-NH$_2$ (SEQ ID NO:1)

PtscGCNle<u>DHF$_d$ RWK</u>-NH$_2$ (SEQ ID NO:30)

AcCGCNle<u>DHF$_d$ RWK</u>-NH$_2$ (SEQ ID NO:31)

DTPA-Nle<u>DHF$_d$ RWK</u>-NH$_2$ (SEQ ID NO:1)

where

Ma is mercaptoacetic acid,

γAbu is γ-aminobutyric acid,

PtscG is 2-(4-phenyl-3-thiosemicarbazidyl)acetic acid, and

DTPA is diethylenetriaminepentaacetic acid.

Page 34, lines 2-3:

Naturally occurring VIP has the sequence:

HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO:2)

Page 34, lines 4-21:

Native VIP is thought to form a helical structure in solution. See Musso *et al. Biochemistry* 27:8174 (1988). The putative helix structure can be stabilized by intramolecular cyclization via the side chains of residues placed in spatial proximity by the helical structure. Examples include:

Ac-HSDAVFTENYTKLRKQNleAAKKYLNDLKKGGT-NH_2 (SEQ ID NO:10)

Ac-HSDAVFTDNYTKLRKQNleAVKKYLNSVLT-NH_2 (SEQ ID NO:32)

(where Nle is norleucine). See O'Donnell *et al. J. Pharm. Exp. Ther.* 270:1282; U.S. Pat. No. 4,822,890; Bolin, Eur. Pat. Appl. 0 536 741 A2. The underlined portion indicates the residues within the cyclized portion of the peptide, and also the termini of the cyclized portion, i.e. the peptide is cyclized via the formation of an amide bond between the side chains of the aspartic acid and the lysine. These cyclic structures are used as a basis for constructing labeled peptides according to the present invention.

Page 34, lines 22-33

Cyclic chelating derivatives based on these structures include, but are not limited to, those with a metal binding moiety attached, either directly or via a spacer group, to the pharmacophore via the side chain amine of a lysine or other bis-amino acid residue. Specific chelating derivatives of this general structure include, but are not limited to:

Ac-HSDAVFTENYTKLRK(PtscGC)QNleAAKKYLNDLKKGGT-NH_2 (SEQ ID NO:10)

(where PtscG=2-(4-phenyl-3-thioseinicarbazidyl)acetic acid); and

Ac-HSDAVFTENYTKLRK(DPTA)QNleAAKKYLNDLKKGGT-NH_2 (SEQ ID NO:10)

(where DTPA=diethylenetriaminepentaacetic acid)

Page 41, lines 3-26 (replace the original table):

Peptide	HPLC[a]	MW[b]
<GHWSYGLRPG-NH_2 (SEQ ID NO:24)	6.1	1183
<GHYSLEWKPG-NH_2 (SEQ ID NO:33)	6.2	1227
HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO:2)	6.7	3326
MaGCγAbuHSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO:2)	7.3	3645
MaGCγAbUVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO:4)	7.5	3235
MaGCγAbuNleDHFR$_d$ WK-NH2[c] (SEQ ID NO:1)	7.0	1302
<GHWSYK(MaGC)LRPG-NH_2 (SEQ ID NO:6)	6.3	1488
<GHYSLK(MaGC)WKPG-NH_2 (SEQ ID NO:7)	6.3	1460
<GHWSYK(Ma-azaGC)LRPG-NH_2 (SEQ ID NO:6)	6.1	1503
<GHYSLK(PtscGC)WKPG-NH_2 (SEQ ID NO:7)	6.9	1536
Ac Nal$_d$ Cpa$_d$ W$_d$ SRK$_d$ (MaGC)LRPA$_d$ –NH_2 (SEQ ID NO:8)	8.2	1668
<GHYSYLK(PtscGDap)WKPG-NH_2 (SEQ ID NO:11)	6.6	1519
<GHYSLK(azaGGC)WKPG-NH_2 (SEQ ID NO:7)	6.5	1474
Nal$_d$ Cpa$_d$ W$_d$ SRK$_d$ (PtscGC)WKPG-NH_2 (SEQ ID NO:12)	8.1	1701
<GHWSYK$_d$ (MaGC)LRPG-NH_2 (SEQ ID NO:13)	6.3	1488
Ac Nal$_d$ Cpa$_d$ W$_d$ SRK$_d$ (AzaGFC)LRPA$_d$ –NH_2 (SEQ ID NO:8)		
Ac Nal$_d$ Cpa$_d$ W$_d$ SRK$_d$ (MaFC)LRPA$_d$ –NH_2 (SEQ ID NO:8)		
Ac Nal$_d$ Cpa$_d$ W$_d$ SRK$_d$ (PtscGC)LRPA$_d$ –NH_2 (SEQ ID NO:8)		
<GHWSYK(iDGDap)LRPG-NH_2 (SEQ ID NO:6)		
<GHWSYK(iECG)LRPG-NH_2 (SEQ ID NO:9)		

Page 42, lines 15-35

Other peptides synthesized by these methods include:

Sequence	MH+	HPLC RT
AcK(TscGC)F_d CFW_d KTCT-OH (SEQ ID NO:14)	1436	7.7
AcK(TscGC)DF_d CFW_d KTCT-OH (SEQ ID NO:15)	1552	7.4
TscGCDF_d CFW_d KTCT-OH (SEQ ID NO:34)	1381	7.7
AcK(TscGC)F_d CFW_d KTCT-ol (SEQ ID NO:14)	1422	7.6
AcK(MtscGC)F_d CFW_d KTCT-ol (SEQ ID NO:14)	1436	7.8
AcK(TscGC)DF_d CFW_d KTCT-ol (SEQ ID NO:15)	1537	7.4
AcK(MaGG)F_d CFW_d KTCT-ol (SEQ ID NO:14)	1378	7.4
K(TscGC)DF_d CFW_d KTCT-NH_2 (SEQ ID NO:15)	1508	7.1
K(TscGC)KKF_d CFW_d KTCT-ol (SEQ ID NO:17)	1651	7.2
K(TscGC)KDF_d CFW_d KTCT-OH (SEQ ID NO:18)	1637	7.3
K(TscGC)DF_d CFW_d KTCT-ol (SEQ ID NO:15)	1495	7.2
K(TscGC)DSF_d CFW_d KTCT-OH (SEQ ID NO:19)	1596	7.4
K(TscGC)DF_d CFW_d KTCT-OH (SEQ ID NO:15)	1508	7.2
K(TscGC)DF_d CFW_d KTCD-NH_2 (SEQ ID NO:20)	1521	7.1
K(TscGC)KDF_d CFW_d KTCT-NHNH (SEQ ID NO:18)	1651	7.2
AcK(TscGC)F_d CFW_d KTCT-$NHNH_2$ (SEQ ID NO:14)	1450	7.4
K(AGC)F_d CFW_d KTCT-ol (SEQ ID NO:14)	1379	6.8
AcK(TscGC)DF_d CFW_d KTCT-ol (SEQ ID NO:15)	1537	7.4
F_d CFW_d KTCTK(TscGC)-NH_2 (SEQ ID NO:21)	1393	6.8

Page 43, lines 1-12:

The method of synthesizing cyclic peptides was demonstrated by preparing the cyclic α-melanocyte stimulating hormone (αMSH) analogue MaGCγ-AbuNleDHF$_d$RWK-NH_2, (SEQ ID NO:1) where the underlining indicates that the peptide sequence is cyclized as a lactam through the

aspartic acid and lysine side chains. The residues to be used for cyclization were side-chain protected as the aloc group (for lysine) and as the allyl ester (for aspartate). The peptide was assembled using Fmoc chemistry as described above, on a polystyrene-based Rink amide resin.

Page 44, lines 5-20:

A Glucoscan (DuPont) vial was reconstituted with 2.18 mCi of $NaTcO_4$ in 1 ml saline to form the ^{99m}Tc-gluceptate complex. <GHWSYK(MaGC)LRPG amide (SEQ ID NO:6) (IMP3) was prepared as above ^{99m} Tc-IMP_3 was prepared by mixing 360 µl (874 uCi) of ^{99m}Tc-gluceptate with 640 µl of peptide in saline. The initially formed precipitate disappeared upon heating for 15 min at 75.degree°. An instant TLC (ITLC) strip developed in H_2O:EtOH:NH_4OH mixture (5:2:1) showed 6.2% of the activity at the origin as colloids. HPLC showed 100% of the activity bound to the peptide with a RT of 6.95 min, whereas the unlabeled peptide eluted at 6.4 min under the same HPLC conditions (reversed phase C-18 column, gradient of 0-100% B in 10 min at a flow rate of 3 ml/min, where A is 0.1% TFA in H_2O and B is 90% CH_3 CN, 0.1% TFA). Recovery from the HPLC column was 85% of the injected activity.

Page 46, lines 3-17(replace the original table)

Peptide	HPLC retention time [a]	HPLC retention time [b]
MaGCγAbuHSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO:2)	7.62 (99%)	7.65
MaGCγAbuVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO: 4)	7.8-9.7[e]	8.19[c] (99%)
<GHWSYK(MaGC)LRPG.amide (SEQ ID NO:6)	6.59 (95%)	6.90[c] (92%)
<GHYSLK(MaGC)WKPG.amide (SEQ ID NO:7)	NA	7.07 (100%)
<GHWSYK(Ma-azaGC)LRPG.amide (SEQ ID NO:6)	6.82 (100%)	7.02[c] (99%)
<GHYSLK(Ptsc-GC)WKPG amide (SEQ ID NO:7)	7.60 (100%)	7.67[d] (100%)
$AcNal_d$ Cpa_d W_d SRK_d (MaGC)LRPA_d – NH_2 (SEQ ID NO:8)	8.50 (27%) 9.00 (68%)	